

# Bison Food Safety Program

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## Tissue Collection Plan

FINAL

1/8/2014

Prepared by:

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Project Abstract: FWS is going to euthanize 11 bison in January 2014 to bring the RMA bison population into balance with available habitat. In order to collect as much scientific information regarding contaminants and contaminant testing protocols from the required population reduction effort, FWS will conduct a targeted tissue collection event in which selected tissues from these bison will be collected and stored for planned and potential future contaminant studies. In addition, a smaller number of animal health surveillance samples will also be collected. This Tissue Collection Plan (TCP) was developed to identify the protocols to be used for collection, preservation, documentation, and storage of selected bison tissues obtained from this necropsy project.

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## NECROPSY TISSUE COLLECTION PLAN (TCP)

### **Purpose**

The primary purpose of this TCP is to collect a wide array of bison tissues that can be used for a variety of studies related to RMA contaminants, including those studies related to bison food safety. The types of studies that may be performed with these tissues include residue analysis for a specific tissue, tissue sample optimization, tissue distribution, inter-laboratory performance comparisons, biopsy methods, and selection of the most appropriate target organ(s) for biomonitoring programs. In addition, the targeted sample collection will provide a training opportunity RMANWR staff that will provide a foundation for future wildlife health training events.

### **Objectives**

The objectives of the TCP are to collect selected bison tissues from each bison in amounts sufficient to support a variety of contaminant study designs. These tissues will be collected so that cross-contamination between tissue types and animals is minimized. The tissues will be preserved by freezing in a manner to ensure a shelf life of at least one year and stored under the requirements outlined in the RMA chain-of-custody program. In addition, selected bison tissues will be collected for animal health surveillance, and will be managed by the R6-Wildlife Health Office.

### **Project Organization**

A table summarizing key staff participating in this study, their organizations, expertise, and primary roles is presented in Table 1 below. David Lucas is responsible for maintaining the official approved version of the TCP.

Table 1. Table of key staff			
<i>Name</i>	<i>Organization</i>	<i>Expertise</i>	<i>Role</i>
Dr. Scott Klingensmith	U.S. Army/Shell	Toxicology	Project Scientist
<b>Team 1</b>			
Lee Jones	USFWS	Wildlife Disease	Necropsy Trainer
Jason Harheine	USFWS	Wildlife Health Technician	Sample Assistant and Photographer
Joe Kilpatrick	Volunteer	Wildlife Biology	Sample Assistant
Ray Fetherman	Volunteer	Operations	Sample Assistant
<b>Team 2</b>			
Tom Ronning	USFWS	Bison Management	Necropsy Lead/ Animal Dispatch
Nick Kaczor	USFWS	Wildlife Biology	Necropsy Assistant



David Lucas	USFWS	Field Biology/ Program Management	Sample Assistant/ Decisionmaker
William Kutosky	USFWS	Wildlife Biology	Sample Assistant/ Animal Transport
<b>Team 3</b>			
Chris Spivey	USFWS	Law Enforcement	Necropsy Lead/ Animal Dispatch
Scott Whiteaker	USFWS	Field Biology	Necropsy Assistant/ Animal Transport
Mindy Hetrick	USFWS	Wildlife Biology	Sample Assistant
Bruce Hastings	USFWS	Wildlife Biology	Sample Assistant
<b>Sample Management</b>			
Brian Fairchild	USFWS	Field Biology	Sample Manager
Abby Wright	USFWS	Wildlife Biology	Sample Mgr Assistant
Gaddy Bergman	Volunteer	Microbial Ecology	Sample Mgr Assistant

## Tissues

The type, portions, number of samples, and approximate total amounts, for each tissue are presented in Table 2.

Table 2. Tissues, Types, Portions, and Amounts to be Collected (as available), excluding wildlife health surveillance samples managed by the R6-Wildlife Health Office

TISSUE	TYPE(s)	PORTIONS	TOTAL	NOTES
Fat (fa)	Coronary (co), pericardial (pc), perirenal (pr), omasal (om) + subcutaneous (sc-tail pad)	5 x 1 g each (01-05) +1 x 30 g – co, pc, sc (06-08) +4 x 30 g pr and om (09-16)	335 g	n=16/ bison
Blood (bl)	Venous (jugular)	3 x 7- 9 ml; whole blood (wh), serum (se), plasma (pl)	~30 ml	n=3/bison
Liver(li)	Central lobe	5 x 30 g + (01-05) 1 x 200 g (06)	350 g	n=6/bison

Kidney (ki)	Right	5 x 30 g + (01-05) 1 x 200 g (06)	350 g	n=6/bison
Brain (br)	Transverse (tv) cut at mid-section	5 x 30 g (01-05)+ 1 x 200 g (06)	350 g	n=6/bison
Muscle (mu)	Tenderloin (tl)	5 x 30 g (01-05) + 2 x 500 g (06-07)	1150 g	n=7/bison
Muscle (mu)	Ribeye (ri)	5 x 30g (01-05) + 2 x 500 (06-07)	1150 g	n=7/bison
Lung (lu)	Right lobe (rl)	5 x 30 g + (01-05) 1 x 200 g (06)	350 g	n=6/bison
Spleen (sp)	Central part of organ	5 x 30 g (01-05)	150 g	n=5/bison
Marrow (ma)	Femur (fm)	5 x 10g (01-05)	50 g	n=5/bison
Hair(ha)	Head (hd)	100 strands (01)	NA	n=1/bison
Fetal Tissue (fe) (as available)	Fat (fa), liver (li), kidney (ki), brain(br), muscle (mu), blood (bl)	Entire organ + 30 g muscle + 30 g fat (combined)	Will vary	Cord blood if available

Total sample n/bison = up to 68 + any available fetal tissues

The following animal health surveillance tissue samples will also be collected:

- Blood – 7 ml EDTA purple top
- Blood – 8.5 ml red top SST
- Deep nasal swab (1)
- Lung (3)
- Liver (2)
- Previously biopsied tail pad area (1)

Any other tissue remarkable for disease or other abnormality will be collected and preserved as appropriate.

### Collection Protocol

Bison will be euthanized by gunshot in the corral area. Once death is confirmed, a blood sample will be collected, followed by a deep nasal swab. The bison will then be transported to the seed barn for necropsy.



An outline of standard full necropsy procedure is provided as Attachment 1. However, this project will use a partial necropsy procedure tailored to a primary focus on targeted tissue collection as directed by the R6-Wildlife Health Biologist. Each bison will have a necropsy team composed of a necropsy lead, necropsy assistant, and 1-2 sample collection assistants. Tissues for contaminant analysis will be collected as soon as they become available during the necropsy procedure. Solid tissues will be excised, handled, and cut using cleaned implements for each animal. Necropsy instruments will be decontaminated with appropriate combinations of soap (Alconox®), water, and alcohol. Small portions of tissues (~30 g) will be placed in glass tissue jar and approximate weight noted.

Large solid tissues will be weighed, wrapped in two layers of aluminum foil, and placed in Whirl-Paks®. All weights are approximate +/- 20%. Blood will be collected with disposable needles and syringes. Plasma and serum samples will be placed on ice after collection, spun down, and frozen.

A box with pre-labeled sample containers and a sample checklist, as well as a second box for transferring samples to the Sample Management station will be assembled for each animal. As samples are collected for each tissue, the samples will be checked off the checklist and placed in the sample transfer box. When all the samples for a tissue have been collected, they will be transferred to the Sample Manager with the completed checklist for sample processing, c-o-c preparation, and storage.

Animal health surveillance tissues will be collected, labeled, preserved, and stored using standard necropsy procedures as directed by the Wildlife Disease Biologist.

Two empty 2oz. sample jars will be opened during each round of necropsies and stored as trip blanks for the laboratory analyses.

## **Documentation, Preservation, Labeling and Packaging and Shipping**

Procedures described in this section are designed to ensure sample integrity through proper sample handling. Samples must be properly handled at the sampling site and during preparation for storage. After approximate weight is obtained and any sample treatment completed (blood), labels and the Chain-of-Custody (c-o-c) form will be properly completed and labels affixed to the sample containers. Information shall be recorded on labels and chain of custody forms using the RMA C-O-C Entry computer program. Labels and c-o-c information may be recorded by hand with a permanent indelible pen if they are legible and complete.

Sample labels shall be completed immediately before or during collection of the corresponding sample. Handwritten labels must contain all required information. Labels shall be securely placed on appropriate sample containers. Label entries include.

- Field Sample ID – 14 + last eight digits of PIT
- Animal Number – 2 letter/number abbreviations for tissue, sub-type, replicate, fetal. (e.g., fasc03fe = third replicate of subcutaneous fat from fetus)
- Date - Year (4 digits)/Month/Day of sample collection.
- Time - Time of sample collection using the 24-hour clock method.
- Container Type - The size and material type of the sample container.
- Site Type – BIOL
- C-O-C Number - The number of the c-o-c corresponding to the label.
- Remarks - Any miscellaneous comments related to sample collection or analysis.

C-o-c records will be used to document the security and control process for samples from the time of collection until delivery to the laboratory. Since the study designs that will use tissues from this collection effort have not been established, no analytical information will be entered at this time. C-o-c entries shall include the following information.

- Contracting Office Representative (COR) POC - The point of contact to resolve sampling issues.
- Project Name – 2014 Bison Necropsy
- Sampling Technique – “0” (necropsy)
- Prime Contractor - FW
- Sampling Program – BIS
- Matrix Code - PT
- Sampler's Initials - The initials of the person or persons collecting the samples.
- Sampler's Signature - The signature of a member of the Sample Management Team that received the samples.
- File Type - CBT (Chemical Bison Tissue)
- Field Sample ID – 14 + last eight digits of PIT
- Site ID – 2 letter/number abbreviations for tissue, sub-type, replicate, fetal
- Site Type - BIOL.
- Sample Date - Year/Month/Day of sample collection.
- Sample Time - Time of sample collection. Time shall be entered using a 24-hour clock method.



- Remarks - Enter any comments or other information needed to identify the sample, such as turnaround time, container size, or preservatives used, if other than ice.

Custody seals will be used to ensure that sample container integrity is not compromised. Custody seals are placed on individual sample containers or on the outside shipping container in such a manner that the container cannot be opened without compromising the custody seal. Once in place, either the sampler or their designee, or the laboratory can break custody seals. In order to transfer custody of the samples, one of the individuals collecting the samples will sign the c-o-c in the first "Relinquished By" box, located under the "Other Notes" box. The date and time of relinquishment is indicated in the "Date" and "Time" boxes. The person receiving the samples shall sign in the adjacent "Received By" box. Note that FedEx does not sign custody forms.

When the sample is placed in a freezer, the date and time of storage is entered in the first date and time boxes and the location is entered in the first "Received By" box. When the sample is removed from storage, the handler will initial the "Relinquished By" box indicating where the sample was received from, the time and date, and sign the "Received By" box following the date.

Samples will be flash frozen with dry ice, and selected tissues placed in lockable -20°C freezers for short-term storage; additional tissues may eventually be placed in a -80°C freezer for long-term storage based on schedule considerations.

Sample collection is scheduled to begin in January 14, 2014 and be completed by January 15, 2014.

## **Health and Safety Plan**

In accordance with 29 CFR § 1910.133, A USFWS Job Hazard Analysis will be conducted prior to the Bison Necropsy Project. In addition, sharps disposal containers will be used to safely dispose of needles and other sharps.

## **Reporting**

A summary report detailing the results of the bison tissue collection effort will be prepared and shared with the Regulatory Agencies within 90 days of sample collections.



## Attachments

## Attachment 1: FWS NECROPSY PROTOCOL

**R6-Wildlife Health Bison Necropsy Protocol: Rocky Mountain Arsenal 2014**

**Version 12.19.13**

*Collect 1 freeze and 1 fix of every target tissue and lesion unless otherwise specified below, including crossing from "normal" into "abnormal" areas; if more than 1 type of lesion in a tissue is found, collect a set for each type of lesion. Photos of everything collected. Samples for histopathology should be 1/16 – 1/8<sup>th</sup> inch thick x 1 inch x 1 inch and should include all appropriate structures. Tissue to formalin ratio in jars = 1:10*

1. Blood should be collected immediately after euthanasia and immediately inserted into Serum Separator (SST) and EDTA vacutainer tubes.
  - a. SST tubes must sit at room temperature for at least 1 hour prior to centrifugation.
  - b. EDTA tubes must immediately be inverted after filling to correct level, then refrigerated within 1 hour. Blood smears must be made within 24 hours of collection.
2. Carcass is positioned in left lateral recumbancy.
3. Conduct external exam – photo and sample as needed.
  - a. Collect histopathology and photographs from chuteside biopsy affected tissues.
4. Collect deep nasal swab and place in media immediately.
5. Examine synovial fluid and coxofemoral joint.
6. Examine ribcage and photograph.
7. Examine organs in place: size, position, matched lesions to ribcage. Photograph total, thoracic and abdominal cavities.
8. Photograph and sample lungs in situ if small lesions are present; then remove to examine and photograph more thoroughly.
  - a. Lungs: palpate and scrape all surfaces clean; photograph.
  - b. Esophagus, trachea: photograph and sample as needed.
  - c. Shred one side lungs and bread slice the other, including exam specifically for parasites; photograph and collect samples.
  - d. Heart: pericardial sac fat, coronary fat, pericardial fluid, pulmonary artery: photograph and sample as needed.
  - e. Examine right heart, check AV and semilunar valves, pulmonary artery adventitia and intima.
    - i. Examine epicardium, myocardium, endocardium – sarcocysts.
    - ii. Examine papillary muscle, trabecular carneae, cordae tendoneae.
  - f. Repeat exam for left heart and aorta.
  - g. Evaluate thickness of left ventricle freewall to right ventricle freewall
9. Renal exam, including fat: photograph and sample.
10. Liver: palpate, examine and evaluate density – collect samples and photograph.
11. GI: Omental fat score
  - a. Examine rumen, reticulum, omasum, abomasum
  - b. Examine duodenum, jejunum, ileum, cecum, large intestine, colon -- photo & sample, including ileocecal junction and ileocecal junction lymph node and mesenteric lymph nodes
12. Examine spleen – sample and photos if needed.
13. Examine bladder – sample and photos if needed.



Final 1.8.14

14. Examine repro for females or if otherwise indicated.

*Animal surveillance samples (with photos and descriptions) for this necropsy:*

1 EDTA blood tube

Deep nasal swab (in media)

Lung (2 freeze, 1 formalin)

Pericardial fluid (1 freeze)

Kidney (1 freeze, 1 formalin)

Liver (2 freeze, 1 formalin)

Tail fat pad

## Attachment 2: Rocky Mountain Arsenal 2014 Necropsy Sampling Form

## USFWS – Wildlife Health Mountain Prairie Region

## Rocky Mountain Arsenal 2014 Necropsy Sampling Form

Name \_\_\_\_\_ Date \_\_\_\_\_ Location Rocky Mountain ArsenalSpecies Bison Age \_\_\_\_\_ Sex \_\_\_\_\_ Site ID \_\_\_\_\_ Eartag \_\_\_\_\_ Pittag \_\_\_\_\_

Pregnancy Status (circle one) Pregnant Not Pregnant NA

Body Condition (circle one) Excellent Good Fair Poor

Carcass Condition<sup>+</sup> (circle one) Excellent Good Fair Poor<sup>+</sup>Excellent = Fresh, mostly intact; Good = Examined within 24 hours of death; Fair = 1-2 days old; Poor = >2 days old; These vary depending on weather.

Comments \_\_\_\_\_

**Sample Checklist (health surveillance samples indicated with a \*):**

_____ *Deep nasal swab (Mycoplasma media)	_____ Kidney: 5 @ 30 grams
_____ *purple top EDTA	_____ Kidney: 1 @ 200 grams
_____ *red/gray top SST	_____ *Liver: 2 histo
_____ Blood tube: 1 no add(red top) (8 ml)	_____ Liver: 5 @ 30 grams
_____ Blood tube: 1 SST (tiger top) (8.5 ml)	_____ Liver: 1 @ 200 grams
_____ Blood tube: 1 EDTA (purple top) (7 ml)	_____ Omasal fat: 1 @ 1 gram
_____ Hair from head: 100 strands	_____ Omasal fat: 4 @ 30 grams (or whatever remaining)
_____ *Tailhead Biopsy: gross description	_____ Spleen: 5 @ 30 grams
_____ *Tailhead Biopsy: photos	_____ Fetal blood: whatever possible
_____ *Tailhead Biopsy: 1 histo	_____ Fetal muscle: 1 @ 30 grams (or whatever possible combined)
_____ Subcutaneous fat- left tailhead: 1 @ 1 gram	_____ Fetal fat: 1 @ 30 grams (or whatever possible combined)
_____ Subcutaneous fat -right tailhead: 1 @ 30 gram (or whatever remaining)	_____ Fetal kidney: entire organ
_____ Marrow from section of femur: 5 @ 10 grams	_____ Fetal liver: entire organ
_____ *Lung: 1 Mycoplasma media	_____ Fetal brain: entire organ (or as much as possible)
_____ *Lung: 1 RC whirlpak	_____ Tenderloin muscle: 5 @ 30 grams
_____ *Lung: 1 histo	_____ Tenderloin muscle: 2 @ 500 grams
_____ Lung: 5 @ 30 grams	_____ Steak muscle (ribeye): 5 @ 30 grams
_____ Lung: 1 @ 200 grams	_____ Steak muscle (ribeye): 2 @ 500 grams
_____ Pericardial fat: 1 @ 1 gram	_____ Brain (transverse mid-section): 5 @ 30 grams
_____ Pericardial fat: 1 @ 30 grams (or whatever remaining)	_____ Brain (transverse mid-section: 1 @ 200 grams
_____ Coronary fat: 1 @ 1 gram	
_____ Coronary fat: 1 @ 30 grams (or whatever remaining)	
_____ Perirenal fat: 1 @ 1 gram	_____ *blood smear to be done at end of day
_____ Perirenal fat: 4 @ 30 grams	_____ *serum spun and poured into cryovials at end of day (1SST)



# EPA Proposed COPC List for Bison Tissues Evaluation

January 8, 2014

Bison Tissue Proposed COPC List 1/8/14	Tox Value(s)	Chemical Index 1986 (666)	Target Comprehensive List of Chemicals included in the August 1988 RMA Chemical Index (320)	HHEA COC List 1990 (64)	Biota RI COC List (39)	Biota RI Major COC List (7)	ROD Human Health COC List for Soil 1996 (27)
1,2,3,4,7,7- Hexachlorobicyclo (2.2.1)hepta-2,5-diene	N	1,2,3,4,7,7- Hexachlorobicyclo (2.2.1)hepta-2,5-diene	1,2,3,4,7,7- Hexachlorobicyclo (2.2.1)hepta-2,5-diene				
4,5,6,7,8,8-Hexachloro- 3a,4,7,7a-tetrahydro-4,7- methano-1H-indene	N	4,5,6,7,8,8-Hexachloro- 3a,4,7,7a-tetrahydro-4,7- methano-1H-indene	4,5,6,7,8,8-Hexachloro- 3a,4,7,7a-tetrahydro-4,7- methano-1H-indene				
Aldrin	Y	Aldrin	Aldrin	Aldrin	Aldrin	Aldrin	Aldrin
Chlordane	Y	Chlordane	Chlordane	Chlordane	Chlordane		Chlordane
DDD	Y	DDD	p,p'-TDE				
DDE	Y	DDE	DDE	DDE	DDE	DDE	DDE
DDT	Y	DDT	DDT	DDT	DDT	DDT	DDT
Dieldrin	Y	Dieldrin	Dieldrin	Dieldrin	Dieldrin	Dieldrin	Dieldrin
Endrin	Y	Endrin	Endrin	Endrin			Endrin
Heptachlor	Y	Heptachlor	Heptachlor		Heptachlor		
Heptachlor epoxide	Y	Heptachlor epoxide	Heptachlor epoxide		Heptachlor epoxide		
Hexachlorobenzene	Y	Hexachlorobenzene	Hexachlorobenzene				
Hexachlorobutadiene	Y	Hexachlorobutadiene	Hexachlorobenzene				
Hexachlorocyclopentadiene	Y	Hexachlorocyclopentadiene	Hexachlorocyclopentadiene	Hexachlorocycl opentadiene			Hexachlorocycl opentadiene
Isodrin	N	Isodrin	Isodrin	Isodrin	Isodrin	Isodrin	Isodrin
Oxychlordane	N	Oxychlordane					
Octachlorocyclopentene	N	Octachlorocyclopentene					
Pentachlorobenzene	Y	Pentachlorobenzene					
Pentachlorophenol	Y	Pentachlorophenol					
Mercury	Y	Mercury	Mercury	Mercury	Mercury	Mercury	Mercury